Preparation of Encapsulated Magnetite Microparticles with Hydroxyapatite

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Preparation of encapsulated magnetite microparticles with hydroxyapatite

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Abstract

Apatite Nuclei were synthesized by raising pH of simulated body fluid (SBF). The Apatite Nuclei were attached to the surfaces of magnetite microparticles. By the soak in SBF, hydroxyapatite was induced from the Apatite Nuclei and covered the whole surface area of the magnetite microparticles, and then encapsulated magnetite microparticles with hydroxyapatite were fabricated. The encapsulated magnetite microparticles with hydroxyapatite were soaked in saline and changes in Fe concentration in saline were measured. Hydroxyapatite encapsulating magnetite microparticles inhibited elution of Fe from magnetite.

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Keywords: Apatite Nucleus; Biomimetic method; Hydroxyapatite; Hyperthermia; Magnetite; Microcapsule; Simulated body fluid

1. Introduction

Hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) is a main inorganic component of our bone and has attracted much attention as a biomaterial with high bioaffinity. It has high affinity to living bone [1-4] and cells [5,6] and an ability to absorb biopolymer such as protein [7]. From these properties, hydroxyapatite is considered as one of the most important biomaterials.

In living body, most of artificial materials are covered with fibrous tissue by an immune reaction of living body and isolated from surrounding tissue [8]. However, hydroxyapatite forms bonelike apatite layer on their surface in living body [9,10]. As a result, hydroxyapatite can avoid the immune reaction and tightly bond to the surrounding living bone thorough this bonelike apatite layer.

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Kokubo et al. invented a simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma [11-13]. It became possible to reproduce the reaction of bonelike apatite formation in living body. Kokubo, Yao and Tanahashi applied the biomimetic reaction in SBF and formed hydroxyapatite thin film on the surface of various kinds of substrates [14-20].

When the pH or the temperature of SBF is raised, fine particles of calcium phosphate are precipitated. Recently, we discovered that the fine particle actively induces hydroxyapatite either in SBF or in body fluid and we named the particle Apatite Nucleus [21].

We presented that hydroxyapatite microcapsules can be fabricated by using biomimetic method [22,23]. The method is as follows. For the first process, Apatite Nuclei are attached to the surfaces of microparticles. For the second process, the microparticles are soaked in SBF. By this treatment, hydroxyapatite is induced from the Apatite Nuclei and grows over the whole surface area of the microparticles. As a result, hydroxyapatite is coated on the whole surface of the microparticles and hydroxyapatite microcapsules can be obtained. By this method, it is expected to encapsulate various kinds of microparticles with hydroxyapatite.

In the previous studies, by using Apatite Nuclei, we fabricated hollow hydroxyapatite microcapsules [24], encapsulated silicagel microspheres with hydroxyapatite [25] and hydroxyapatite microcapsules containing insulin in silicagel microspheres [26]. The hydroxyapatite microcapsules possess high bioaffinity and are thought to be useful to drug delivery systems. For the hydroxyapatite microcapsules containing insulin in silicagel microspheres, sustained-release of insulin was achieved.

Hyperthermia is one of the most attractive methods for cancer therapy which can selectively heat in tumors and kill cancer cells with minimum damage to surrounding normal tissue. Magnetite is a typical ferrimagnetic material and is thought to be useful to cancer hyperthermia [27]. Hydroxyapatite microcapsules are thought to be able to provide bioactivity to ferrimagnetic materials and useful to hyperthermia for bone cancer.

In the present study, we encapsulated magnetite microparticle with hydroxyapatite by biomimetic method using Apatite Nuclei.

2. Materials and Methods

2.1. Preparation of SBF

SBF was prepared by dissolving reagent-grade sodium chloride (NaCl: Wako Pure Chemical Industries, Japan), sodium hydrogen carbonate ($NaHCO_3$: Hayashi Pure Chemical Ind., Japan), potassium chloride (KCl: Hayashi Pure Chemical Ind., Japan), di-potassium hydrogen phosphate trihydrate ($K_2HPO_4 \cdot 3H_2O$: Nacalai Tesque, Japan), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$: Hayashi Pure Chemical Ind., Japan), calcium chloride ($CaCl_2$: Hayashi Pure Chemical Ind., Japan) and sodium sulfate (Na_2SO_4: Hayashi Pure Chemical Ind., Japan) in ultrapure water with the composition as shown in Table 1 and buffered at pH 7.40 with tris(hydroxymethyl)aminomethane ($(CH_2OH)_3CNH_2$: Hayashi Pure Chemical Ind., Japan) and 1 $mol \cdot dm^{-3}$ hydrochloric acid (HCl: Hayashi Pure Chemical Ind., Japan) at 36.5 °C.

2.2. Precipitation of Apatite Nuclei

The pH of SBF was raised to pH 8.60 by dissolving $(CH_2OH)_3CNH_2$ at 25.0 °C. By this treatment, we precipitated Apatite Nuclei in the SBF. Apatite Nuclei were collected by filtration using a

Table 1. Ion concentrations of simulated body fluid (SBF) and human blood plasma.

	Ion concentration / mM	
	SBF	Blood plasma
NaCl	142.0	142.0
$NaHCO_3$	5.0	5.0
KCl	2.5	2.5
$K_2HPO_4 \cdot 3H_2O$	1.5	1.5
$MgCl_2 \cdot 6H_2O$	147.8	103.0
1 M HCl	4.2	27.0
$CaCl_2$	1.0	1.0
Na_2SO_4	0.5	0.5

polytetrafluoroethylene membrane filter with 50 nm for average pore size (Millipore, USA), washed with distilled water, and dried at 36.5 °C. Apatite Nuclei were dispersed in ultrapure water with ultrasonic vibration, and Apatite Nuclei-dispersed solution was obtained.

2.3. Preparation of encapsulated magnetite microparticles with hydroxyapatite

1 mg of commercially obtained magnetite microparticles (Kojundo Chemical Laboratory, Japan) were soaked in the Apatite Nuclei-dispersed solution mentioned above and dispersed with ultrasonic vibration. By this treatment, Apatite Nuclei were attached to the surface of the magnetite microparticles. The magnetite microparticles were collected by filtration. These magnetite microparticles were soaked in SBF at pH 7.40 at 36.5 °C for 7 d. After that, the magnetite microparticles were collected by filtration, washed with ultrapure water, and dried at 36.5 °C.

2.4. Observation of the microparticles

Thus obtained magnetite microparticles were analyzed by scanning electron microscopy (SEM: ESEM-2700, Nikon, Japan) and energy dispersive X-ray analysis (EDX: DX-4, EDAX International, USA). In SEM observation, we conducted Au sputtering on the surface of the specimen. For the reference, the not-encapsulated magnetite microparticles were also analyzed by SEM and EDX.

2.5. Evaluation of Fe elution

1 mg of the encapsulated magnetite microparticles with hydroxyapatite were soaked in 10 cm^3 saline (0.01 $mol \cdot dm^{-3}$ phosphate buffered saline, pH at 25 °C: 7.2-7.4, Wako Pure Chemical Industries, Japan). The saline was continued to shake by using shaking apparatus for up to 168 h in an incubator held at 36.5 °C. Changes in Fe concentration in saline were measured by inductively coupled plasma atomic emission spectroscopy (ICP: ICPS-7500, Shimadzu, Japan). For the reference, not-encapsulated magnetite microparticles were also dispersed in saline and conducted the same measurement.

3. Results and Discussion

3.1. Observation of the encapsulated magnetite microparticles with hydroxyapatite

Fig. 1 shows (a) SEM micrograph and (b) EDX profile of the not-encapsulated magnetite microparticles. In Fig. 1(a), it was observed that the not-encapsulated magnetite microparticles was about 300 nm in particle size. In Fig. 1(b), no peak other than Fe and O due to magnetite except C due to a carbon tape and Au due to gold sputtering in SEM observation process was detected.

Fig. 2(a) shows SEM micrograph of the magnetite microparticle soaked in the Apatite Nuclei-dispersed solution, and then soaked in SBF for 7 d of low magnification. In SEM observation, many magnetite microparticles were encapsulated with hydroxyapatite were observed. Fig. 2 (b) shows EDX profile of the magnetite microparticles shown in Fig. 2(a). Peaks of phosphorous (P) and calcium (Ca), constituents of hydroxyapatite, were detected on the surface. Also, a peak of Fe, constituents of magnetite, was also observed. These results indicate that this method has high reproducibility.

Fig. 3 shows (a) picture of a microcapsule by magnification and (b) EDX profile of the magnetite microparticles soaked in the Apatite Nuclei-dispersed solution, and then soaked in SBF for 7 d. In Fig. 3(a), it was observed that needle-like crystallites characteristic to hydroxyapatite coated whole surface of the magnetite microparticles. In Fig. 3(b), peaks of P and Ca, constituents of hydroxyapatite, were detected on the surface. Also, a peak of Fe, constituents of magnetite, was also observed. These results indicate that hydroxyapatite was induced from the Apatite Nuclei attached to the surface of the magnetite microparticles and spread over whole surface area of the magnetite microparticles in SBF.

3.2. Fe elution in saline

Fig. 4 shows cumulative elution of Fe from encapsulated magnetite microparticles with hydroxyapatite and not-encapsulated magnetite microparticles in saline up to 168 h at 36.5 °C. In Fig. 4, (a), solid circle and full line, shows the change in elution ratio of Fe for the encapsulated magnetite microparticles and (b), open circle and dotted line, shows that for the not-encapsulated ones. The elution ratio of Fe was calculated by dividing the weight of Fe eluted from magnetite microparticles in saline measured by ICP by that contained in magnetite microparticles soaked in the saline. The elution ratio of Fe for the

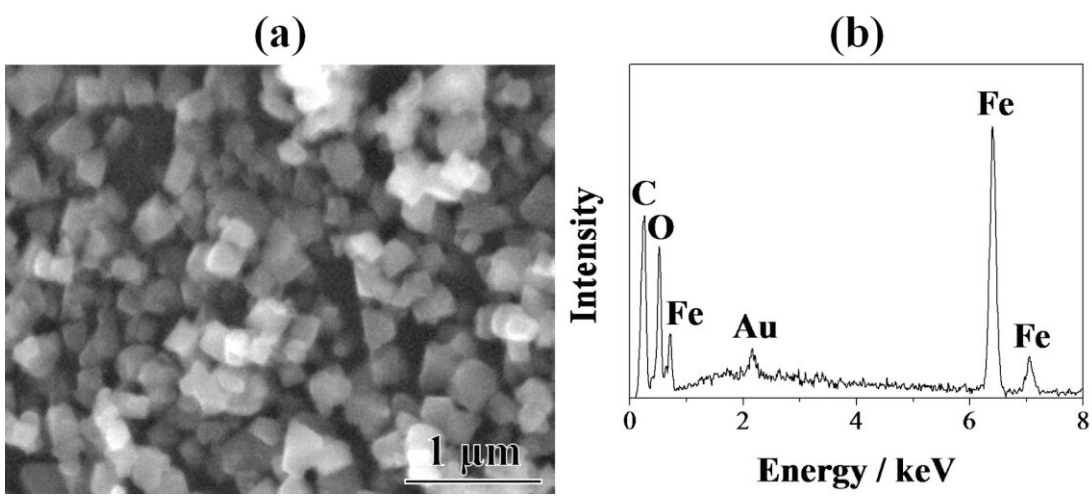

Fig. 1. (a) SEM micrograph and (b) EDX profile of the not-encapsulated magnetite microparticles.

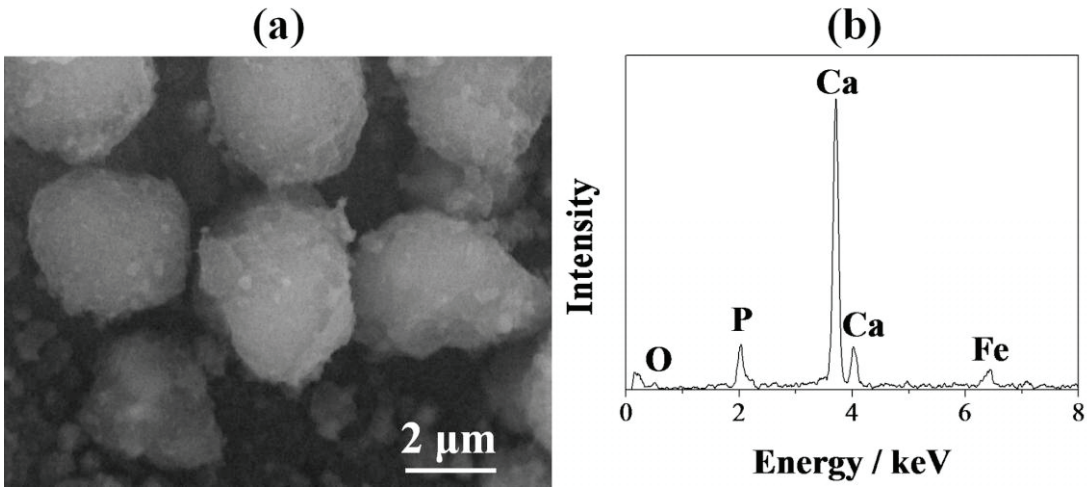

Fig. 2. (a) SEM micrograph of low magnification and (b) EDX profile of the magnetite microparticles soaked in Apatite Nuclei dispersed-solution, and then soaked in SBF for 7 d.

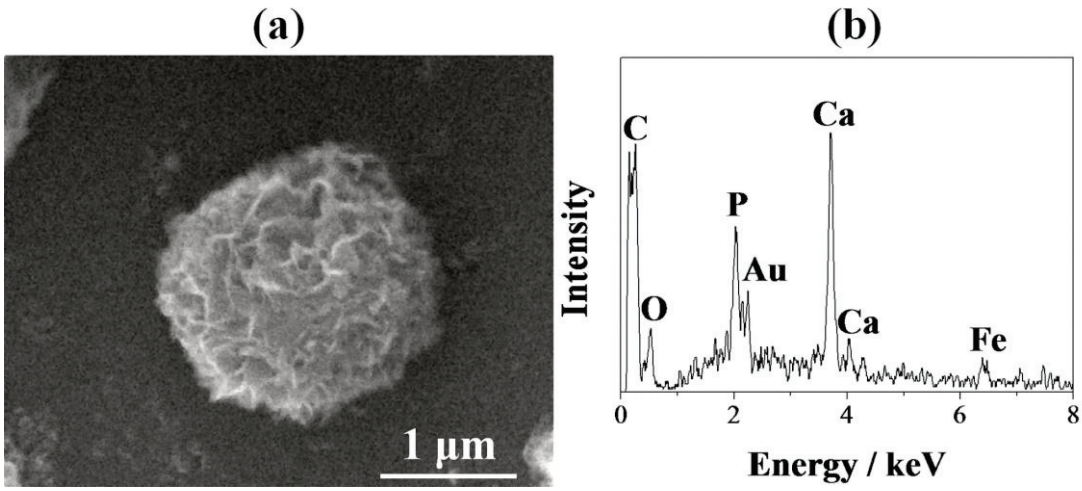

Fig. 3. (a) SEM micrograph and (b) EDX profile of the magnetite microparticles soaked in Apatite Nuclei dispersed-solution, and then soaked in SBF for 7 d.

encapsulated magnetite microparticles with hydroxyapatite was approximately one over ten of that for not-encapsulated ones. This result indicates that hydroxyapatite encapsulating magnetite microparticles inhibit elution of Fe from magnetite.

4. Conclusion

When the pH or the temperature of SBF is raised, fine particles of calcium phosphate are precipitated in the fluid. We found that these particles are very active for forming hydroxyapatite from SBF and we named this particle Apatite Nucleus.

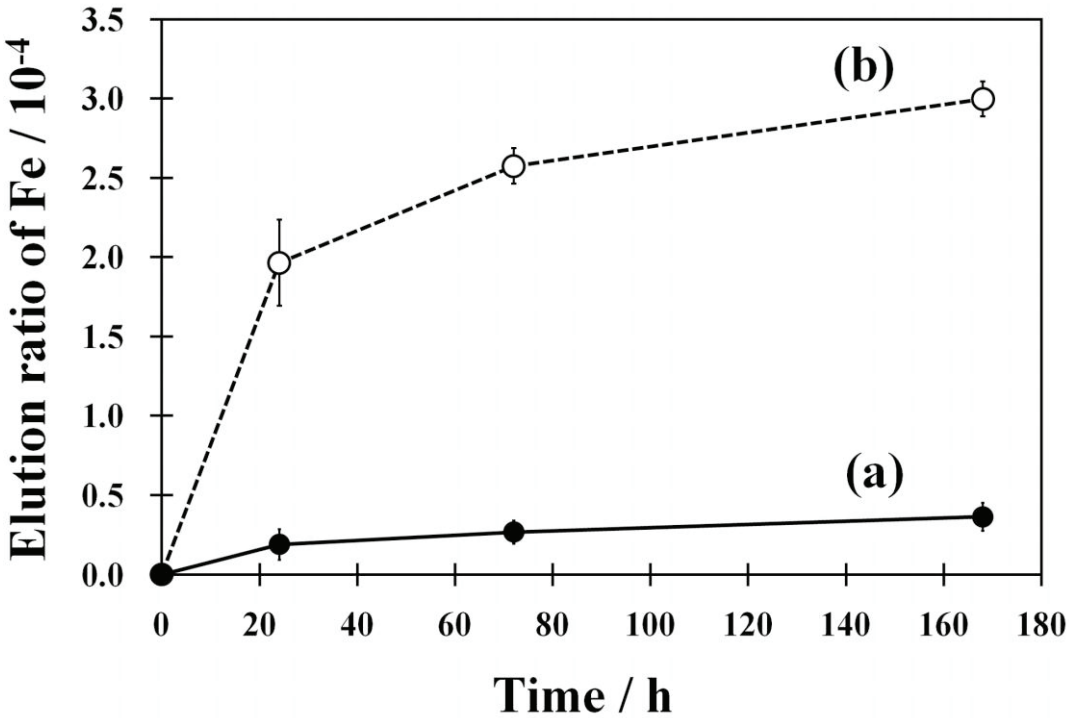

Fig. 4. Cumulative elution of Fe from (a) encapsulated magnetite microparticles with hydroxyapatite (solid circle and full line) and (b) not-encapsulated magnetite microparticles (open circle and dotted line) in saline up to 168 h at 36.5 °C.

We have successfully fabricated encapsulated magnetite microparticles with hydroxyapatite by biomimetic method. Apatite Nuclei were synthesized by raising pH of SBF. Hydroxyapatite was formed from Apatite Nuclei attached on the magnetite microparticles by soaking in SBF, and then the encapsulated magnetite microparticles with hydroxyapatite were obtained. Hydroxyapatite encapsulating magnetite microparticles inhibit elution of Fe from magnetite. Application of this material is promising for cancer hyperthermia.

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